Amendments to the Claims

This listing of claims replaces all prior versions and listings of claims in the application.

1. **(Previously presented)** A recombinant method for identifying a bioactive peptide comprising:

(a) transforming a host cell with an expression vector comprising a tightly regulable control region operably linked to a nucleic acid sequence encoding a peptide;

(b) growing the transformed cell under conditions that repress expression of the peptide;

(c) inducing expression of the peptide in the transformed host cell; and

(d) determining whether expression of the peptide is inhibitory of host cell growth, wherein inhibition of host cell growth is indicative of the expression of a bioactive peptide.

2. - 60. **(Canceled)**

61. **(Withdrawn)** The method of claim 1 wherein the tightly regulable control region of the expression vector comprises at least a portion of the wild-type *E. coli lac* promoter/operator region, said portion comprising auxiliary *lac* operator O3, a CAP binding region, the –35 *lac* promoter site, the –10 *lac* promoter site, *lac* operator O1, *lacZ* Shine-Dalgarno sequence and a spacer region; and wherein the transformed host cell comprises an amount of Lac repressor protein effective to repress expression of the peptide during step (b).

62. **(Withdrawn)** The method of claim 61 wherein the host cell is a bacterium.

63. **(Withdrawn)** The method of claim 62 wherein the bacterium is a gram positive bacterium.

64. **(Withdrawn)** The method of claim 62 wherein the bacterium is gram negative bacterium.

65. **(Withdrawn)** The method of claim 62 wherein the bacterium is *E. coli*.

66. **(Withdrawn)** The method of claim 61 wherein the host cell is a microbial pathogen.

67. **(Withdrawn)** The method of claim 66 wherein the microbial pathogen is a member of a genus selected from the group consisting of *Streptococcus*, *Staphylococcus* and *Enterococcus*.

68. **(Withdrawn)** The method of claim 61 wherein the expression vector comprising the nucleic acid sequence encoding the peptide is a first expression vector, and wherein the host cell is further transformed, prior to step (b), with a second expression vector comprising a promoter operably linked to a gene encoding a Lac repressor protein.

69. **(Withdrawn)** The method of claim 61 wherein the expression vector has the identifying characteristics of pLAC11 (ATCC No. 207108).

70. **(Withdrawn)** The method of claim 69 wherein the expression vector is pLAC11 (ATCC No. 207108).

71. **(Withdrawn)** The method of claim 1 wherein the host cell comprises proteases or peptidases or both.

72. **(Withdrawn)** The method of claim 1 wherein the host cell has not been modified to reduce or eliminate the expression of naturally expressed proteases or peptidases.

73. **(Withdrawn)** The method of claim 1 wherein the host cell is a prokaryote.

74. **(Withdrawn)** The method of claim 1 wherein the host cell is a microbial pathogen.

75. **(Withdrawn)** The method of claim 74 wherein the microbial pathogen is a member of a genus selected from the group consisting of *Streptococcus*, *Staphylococcus* and *Enterococcus*.

76. **(Withdrawn)** The method of claim 1 wherein the host cell is a eukaryotic cell.

77. **(Withdrawn)** The method of claim 76 wherein the eukaryotic cell is a mammalian cell.

78. **(Withdrawn)** The method of claim 76 wherein the eukaryotic cell is a cancer cell.

79. **(Withdrawn)** The method of claim 1 wherein the host cell is a protozoan.

80. **(Withdrawn)** The method of claim 1 wherein the peptide comprises a first

stabilizing group comprising the N-terminus of the peptide and a second stabilizing group comprising the C-terminus of the peptide.

81. **(Withdrawn)** The method of claim 80 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and –Pro-Pro-Xaa.

82. **(Withdrawn)** The method of claim 81 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.

83. **(Withdrawn)** The method of claim 1 wherein the peptide comprises a stabilizing motif.

84. **(Withdrawn)** The method of claim 83 wherein the stabilizing motif comprises a hydrophilic α-helix motif.

85. **(Withdrawn)** The method of claim 83 wherein the stabilizing motif comprises an opposite charge ending motif.

86. **(Withdrawn)** The method of claim 1 wherein the peptide comprises a randomized amino acid sequence.

87. **(Withdrawn)** The method of claim 86 wherein the peptide comprises a first stabilizing group comprising the N-terminus of the peptide and a second stabilizing group comprising the C-terminus of the peptide.

88. **(Withdrawn)** The method of claim 86 wherein the peptide comprises a stabilizing motif.

89. **(Currently amended)** A non-naturally occuring polypeptide comprising a bioactive peptide, ~~comprising~~ a first stabilizing group ~~comprising~~ attached to the N-terminus of the bioactive peptide, and a second stabilizing group ~~comprising~~ attached to the C-terminus of the bioactive peptide, wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-, and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and –Pro-Pro-Xaa~~, with the proviso that when the first stabilizing group is Pro-, the second stabilizing group is not Pro-Xaa~~.

90. **(Currently amended)** The ~~bioactive peptide~~ polypeptide of claim 89 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein, and glutathione reductase.

91. **(Currently amended)** The ~~bioactive peptide~~ polypeptide of claim 89 wherein the first stabilizing group is Pro-Pro- and the second stabilizing group is –Pro-Pro.

92. **(Currently amended)** The ~~bioactive peptide~~ polypeptide of claim 89 wherein at least one of the first and second stabilizing groups comprises a small stable protein.

93. **(Currently amended)** The ~~bioactive peptide~~ polypeptide of claim 92 wherein the small stable protein is a four-helix bundle protein.

94. **(Currently amended)** The ~~bioactive peptide~~ polypeptide of claim 92 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein, and glutathione reductase.

95. **(Currently amended)** The ~~bioactive peptide~~ polypeptide of claim 94 wherein the small stable protein is Rop protein.

96. **(Currently amended)** The ~~bioactive peptide~~ polypeptide of claim 89 which is an antimicrobial peptide.

97. **(Currently amended)** The ~~bioactive peptide~~ polypeptide of claim 89 which is a therapeutic peptide drug.

98. **(Withdrawn)** A bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide.

99. **(Withdrawn)** A fusion protein comprising a four-helix bundle protein and a polypeptide.

100. **(Withdrawn)** The fusion protein of claim 99 wherein the four-helix bundle protein is Rop protein.

101. **(Withdrawn)** The fusion protein of claim 100 wherein the polypeptide comprises a bioactive peptide.

102. **(Withdrawn)** The fusion protein of claim 100 wherein the four-helix bundle protein is covalently linked at its C-terminus to the N-terminus of the polypeptide.

103. **(Withdrawn)** The fusion protein of claim 100 wherein the four-helix bundle protein is covalently linked at its N-terminus to the C-terminus of the polypeptide.

104. **(Currently amended)** A non-naturally occurring polypeptide comprising:

a bioactive peptide ~~comprising (a)~~ ;

a first stabilizing group attached to the N-terminus of said bioactive peptide, wherein said first stabilizing group is selected from the group consisting of a small stable protein, -Pro-, -Pro-Pro-, -Xaa-Pro- and -Xaa-Pro-Pro- [[, and (b)]] ;

a second stabilizing group attached to the C-terminus of said bioactive peptide, wherein said second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and –Pro-Pro-Xaa; and

a cleavage site immediately preceding the first stabilizing group [[:]]

~~wherein the second stabilizing group comprises the C-terminus of the polypeptide~~.

105. **(Currently amended)** A non-naturally occurring polypeptide comprising:

a bioactive peptide ~~comprising (a)~~ ;

a first stabilizing group attached to the N-terminus of said bioactive peptide, wherein said first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro- [[, and (b)]] ;

a second stabilizing group attached to the C-terminus of said bioactive peptide, wherein said second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and –Pro-Pro-Xaa; and

a cleavage site immediately following the second stabilizing group [[:]]-

~~wherein the first stabilizing group comprises the N-terminus of the polypeptide~~.

106. **(Withdrawn)** A polypeptide comprising:

a bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide; and

a cleavage site immediately preceding the plurality of sequential uniformly charged amino acids.

107. **(Withdrawn)** A polypeptide comprising:

a bioactive peptide comprising a plurality of sequential uniformly charged amino acids comprising the N-terminus of the bioactive peptide and a plurality of sequential oppositely charged amino acids comprising the C-terminus of the bioactive peptide; and

a cleavage site immediately following the plurality of sequential oppositely charged amino acids.

108. **(Withdrawn)** A method for using an antimicrobial peptide comprising:

covalently linking a first stabilizing group to the N-terminus of the antimicrobial peptide and a second stabilizing group to the C-terminus of the antimicrobial peptide to yield a stabilized antimicrobial peptide; and

contacting a microbe with the stabilized antimicrobial peptide.

109. **(Withdrawn)** The method of claim 108 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and –Pro-Pro-Xaa.

110. **(Withdrawn)** The method of claim 108 wherein the first stabilizing group is selected from the group consisting of Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro- and the

second stabilizing group is selected from the group consisting of -Pro, -Pro-Pro, -Pro-Xaa and –Pro-Pro-Xaa.

111. **(Withdrawn)** A method for using an antimicrobial peptide comprising:

covalently linking a plurality of sequential uniformly charged amino acids to the N-terminus of the antimicrobial peptide and covalently linking a plurality of sequential oppositely charged amino acids to the C-terminus of the antimicrobial peptide to yield a stabilized antimicrobial peptide; and

contacting a microbe with the stabilized antimicrobial peptide.

112. **(Withdrawn)** A method for treating a patient having a condition treatable with a peptide drug comprising administering to the patient a stabilized form of the peptide drug.

113. **(Withdrawn)** The method of claim 112 wherein the stabilized form of the peptide drug comprises a first stabilizing group comprising the N-terminus of the peptide drug and a second stabilizing group comprising the C-terminus of the peptide drug.

114. **(Withdrawn)** The method of claim 113 wherein the first stabilizing group is selected from the group consisting of a small stable protein, Pro-, Pro-Pro-, Xaa-Pro- and Xaa-Pro-Pro-; and wherein the second stabilizing group is selected from the group consisting of a small stable protein, -Pro, -Pro-Pro, -Pro-Xaa and –Pro-Pro-Xaa.

115. **(Withdrawn)** The method of claim 114 wherein the small stable protein is a four-helix bundle protein.

116. **(Withdrawn)** The method of claim 114 wherein the small stable protein is selected from the group consisting of Rop protein, glutathione sulfotransferase, thioredoxin, maltose binding protein and glutathione reductase.

117. **(Withdrawn)** The method of claim 113 further comprising, prior to administration of the stabilized form of the peptide drug, covalently linking the first stabilizing group to the N-terminus of a peptide drug and covalently linking the second stabilizing group to the C-terminus of the peptide drug to yield the stabilized form of the peptide drug.

118. **(Withdrawn)** The method of claim 112 wherein the stabilized form of the peptide drug comprises an opposite charge ending motif.

119. **(Withdrawn)** The method of claim 118 further comprising, prior to administration of the stabilized form of the peptide drug, covalently linking a plurality of sequential uniformly charged amino acids to the N-terminus of the peptide drug and covalently linking a plurality of sequential oppositely charged amino acids comprising the C-terminus of the peptide drug to yield the stabilized form of the peptide drug.

120. **(Currently amended)** A <u>non-naturally occurring</u> polypeptide comprising a bioactive peptide and a stabilizing group attached to either or both of the N-terminus or C-terminus of the bioactive peptide, wherein the stabilizing group attached to the N-terminus, if present, comprises Xaa-Pro-Pro-, and the stabilizing group attached to the C-terminus, if present, comprises –Pro-Pro-Xaa.

121. **(Currently amended)** A non-naturally occurring polypeptide comprising a bioactive peptide and a stabilizing group comprising Rop protein attached to either or both of the N-terminus or C-terminus of the bioactive peptide.

122. **(Currently amended)** A non-naturally occurring polypeptide comprising a bioactive peptide and a stabilizing group comprising a four-helix bundle protein attached to either or both of the N-terminus or C-terminus of the bioactive peptide.

123. **(New)** The polypeptide of claim 89 wherein the bioactive peptide is a naturally occurring bioactive peptide.

124. **(New)** The polypeptide of claim 104 wherein the bioactive peptide is a naturally occurring bioactive peptide.

125. **(New)** The polypeptide of claim 105 wherein the bioactive peptide is a naturally occurring bioactive peptide.

126. **(New)** The polypeptide of claim 120 wherein the bioactive peptide is a naturally occurring bioactive peptide.

127. **(New)** The polypeptide of claim 121 wherein the bioactive peptide is a naturally occurring bioactive peptide.

128. **(New)** The polypeptide of claim 122 wherein the bioactive peptide is a naturally occurring bioactive peptide.